Building Soft Nanomachines from Polydiacetylene Liposomes

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What is a "soft nanomachine"?

Everyone is familiar with the general concept of a "machine", but a precise definition may be difficult to come by. The chemist, George Whitesides, suggested that a machine is simply "a device for performing a task".[1] We might extend this to say "to be a machine, the object must have been designed and constructed specifically to complete a task and that it consists of multiple distinct parts that operate together to fulfill its function".

To be a "nanomachine", the dimensions of the device must be in the nanoscale size regime. There are varying definitions of this term as well, but here we will define this scale as being between 10 and roughly 200 nanometers across its longest dimension. This is pretty small. A human hair is about 100,000 nanometers wide, while a red blood cell is on the order of 7000 nanometers in diameter. Interestingly, for a chemist, nanosized objects are very large as many of the common molecules used for synthesis are less than one nanometer in length! Nanoscale objects are then either extremely large molecules, such as polymers, or organized assemblies of smaller molecules.

"Soft" is another common term and for our purposes it means "something that is not metallic". In practice, this means the device is made from organic compounds – those that are composed largely of carbon and hydrogen. So, a soft nanomachine is not a submarine or a space satellite that has been somehow shrunken down to the nanoscale, but a device more akin to an enzyme that might operate as part of the machinery inside of a biological cell. Indeed, nature is filled with soft nanomachines and humans can be thought of as enormous collections of nanomachines all working to keep us alive. Biology turns out to be a great source of inspiration for designing nanomachines![2]

This paper describes our journey (perhaps a long and winding one) to design and build soft nanomachines based on a platform that is a spherical assembly of small molecular components.

Polydiacetylenes- A key component

A key element in our efforts to build soft nanomachines is a class of polymers called "polydiacetylenes". Polydiacetylenes (PDAs) are formed from diacetylenes, which are molecules that contain two carbon-carbon triple bonds (acetylenes) separated by a carbon-carbon single bond (see Fig. 1). PDAs are unusual in several ways. Unlike most polymers that are grown in solution as the result of the collision between small molecules called monomers, PDAs are only formed when they react while locked into a highly organized array, such as a crystal. Figure 1 shows how the linear diacetylene molecules must assemble so that they are stacked at about a 45° angle and about 5 angstroms (or ½ of a nanometer) apart.[3] In this figure, the groups "R" can be anything as long as the molecules are able to assemble properly. On exposure to ultraviolet light, gamma radiation or in some cases, just heat, the monomers are transformed to a long polymer chain having a conjugated backbone of the repeating pattern of double bond, single bond, triple bond, single bond, double bond, etc. This backbone is rigid and gives the material (a crystalline solid in this case) a deep blue color.

PDAs and Crystal Engineering

While it had been known for many years that some diacetylenes would become highly colored over time or upon exposure to light, a good explanation of the phenomenon was not developed until the work of Gerhard Wegner in the late 1960s. Wegner placed the reaction in the context of "topotactic transitions" or chemical transformations that take place in crystalline solids.[4] Thus, much of the early interest in PDAs was focused on their important optical and structural properties in crystals.[5] However, in many cases the diacetylene monomers crystalized in

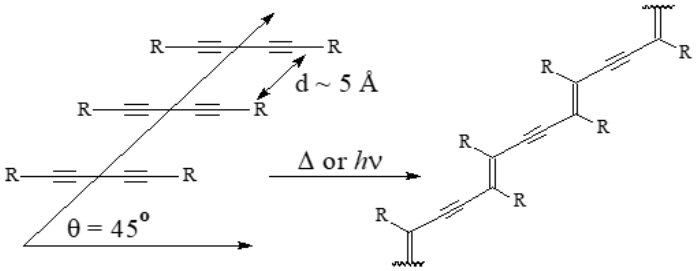


Figure 1: Requirements for the topotactic polymerization of a diacetylene.

geometries that are not suitable for the polymerization reaction to occur, limiting studies to compounds discovered essentially randomly.

The process of designing molecules so that they assemble into crystalline solids that have particular structural features (such as those shown in Fig. 1) is known as "crystal engineering".[6] It requires an understanding of, and control over, the intramolecular chemical interactions that govern crystal formation and stability. There are many of these interactions in any given solid, and they vary in strength and number. The successful prediction of crystalline structure is extraordinarily difficult and currently involves some luck in addition to careful science.

Our initial investigations of polydiacetylenes grew out of our interest in using an unusual intramolecular interaction known as "halogen bonding" for crystal engineering.[7, 8] We had noticed that flat nitrogen-containing aromatic ring compounds, when forming complexes with flat iodine-containing compounds, tended to crystalize in columns with geometries similar to that shown in Fig. 1. These structures can be determined by X-ray crystallography and an example from reference 7 is shown in Figure 2. The dotted lines in this figure indicate the halogen bond between the light blue nitrogen atoms of compound **1** and the large purple iodine atoms of compound **2**. This intramolecular interaction is relatively strong and is instrumental in organizing the solid-state structure of the complex. Many other complexes made from flat molecules were found to assemble similarly.

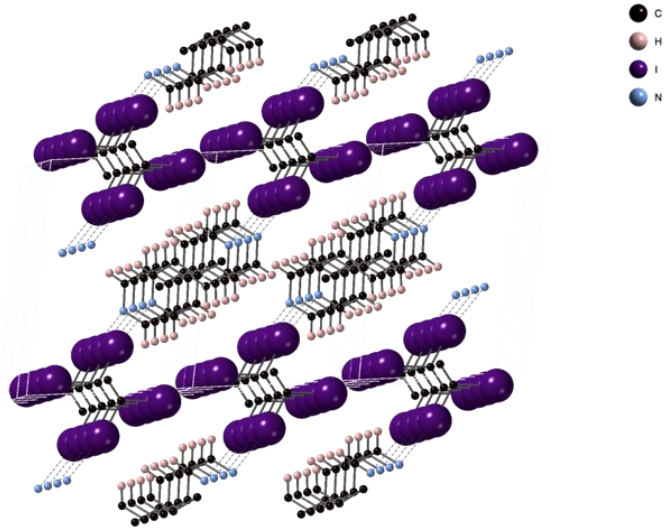
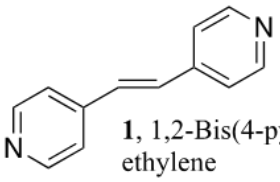
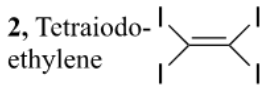


Figure 2: Crystal lattice of 1,2-bis(4-pyridyl)ethylene:tetraiodoethylene.

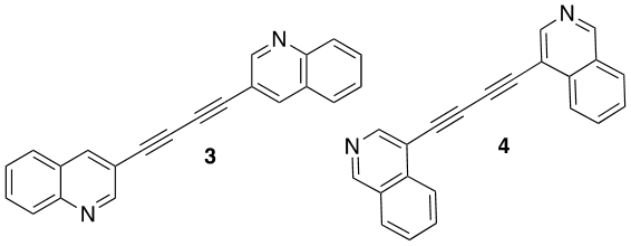


1, 1,2-Bis(4-pyridyl)-ethylene



2, Tetraiodo-ethylene

The organization of these compounds suggested that we might be able to make nanoporous solids, materials with molecular sized channels running through them. By locking the columns of nitrogen-containing molecules (also known as halogen bond acceptors) together, while removing the iodine-containing molecules (or halogen bond donors), we anticipated this might be possible. Nanoporous solids are of interest in several different applications ranging from catalysis to matrices for purification of other compounds. We imagined that compounds containing the nitrogen rings connected via diacetylenes might similarly allow for the formation of columns. Moreover, examples of this were known. Compound **3** had previously been shown to undergo topochemical polymerization, though interestingly, the closely related compound **4** did not.[9]

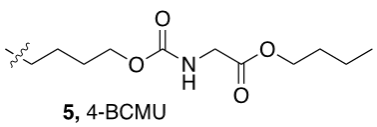


3 **4**

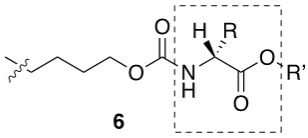
We prepared and crystalized complexes of **3** and **4** halogen bonded to the halogen bond donor compound **2**.[10] Unfortunately, while both assembled into iodine rich and poor stacks, much like the crystalline lattice shown in Fig. **2**, neither of them polymerized. In fact, while we prepared several other members of this family of complexes, none of them were able to undergo the polymerization reaction. From this work, two important observations emerged. First, a reliable molecular feature that would encourage polymerization was required. Second, a closer look at Figure **2** shows that if all of the iodine-containing molecules were removed, the result would not be a nanoporous solid. This is because instead of being organized into self-supporting channels, the two molecules of the composite are actually organized into layers of donors and acceptors. These would necessarily collapse if the donor layers were removed.

Polymerogenic PDAs

We first chose to address the polymerization problem by looking into "polymerogenic" groups, a molecular fragment that could be incorporated into diacetylenes and would encourage the proper alignment for polymerization. The 4-BCMU group (butoxycarbonylmethylurethane, **5**) had been shown by several workers to be an effective diacetylene polymerization agent. Note that the wavy line at the left edge of the fragment indicates the position of attachment to the diacetylene. The hydrogen on the nitrogen interact through hydrogen bonding with the carbonyl of the urethane in order to help guide the crystal to the required geometry.[11]



5, 4-BCMU



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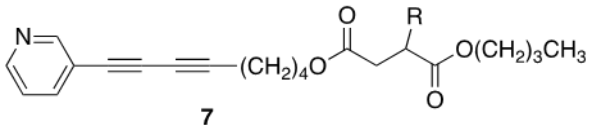
We set out to look for variations on the BCMU framework that might provide additional structural control and to explore the limitations of the strategy. We made a large number of diacetylenes with fragment **6** on both ends of the diacetylene.[12, 13] The R and R' groups could be any of a variety of organic fragments. All of these derivatives retain the hydrogen bonding moiety that was found to be essential to the organization of the compounds for polymerization. However, this ability was affected by both R and R'. Most of the compounds did polymerize to some degree, though the yields and chain length varied from excellent to poor. In addition, the colors of the resulting polymers varied greatly, ranging from blue to red to yellow, which indicate decreasing polymerization efficiency. The chromatic properties of PDAs will be discussed in later sections.

The portion of **6** within the dotted box originated from naturally occurring amino acids. These compounds, the building blocks of proteins, have the property of "chirality", or handedness, at the carbon atom between the nitrogen and the ester carbonyl. To understand the concept of chirality here, note that the group R can either be coming out of the plane of the paper or out the back, as shown in **6**. Natural amino acids only come in the version indicated.[14] Chirality is a feature of many biological molecules and is responsible for the *direction* of the twist in the DNA double helix as well as many other biochemical structural features. The PDAs assembled by this process adopt a particular helical confirmation. Since asymmetrical diacetylenes can easily be prepared, we designed a series that would assemble into a spiral, with halogen bond acceptors pointed inwards. Compound **7** is an example, with a chiral polymerogenic group of type **6** one side and a halogen bond acceptor on the other. Altering the size of the R group alters the pitch of the helix and could allow for the construction of solids composed of helical channels filled with removable halogen bond donors.



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Synthesis of U-Shaped PDAs

Another approach to nanoporous materials is to strengthen the pores against collapse once the guest molecule, a halogen bond donor in these cases, is removed. Through a combination of chemical intuition and computer modeling, structures of type **12** were designed and the synthetic scheme shown in Fig. **3** was proposed.[15] The colored circles were either pyridine or thiophene and the letters X and Y were bromine and iodine, respectively. BCMU was the one of the polymerogenic groups discussed in the previous section. Depending on which ring was used as the blue base unit and which was used in the red arm unit, the cavity size would change slightly, but each would be capable of making three halogen bonds (indicated by arrows in structure **12**) with a halogen bond donor guest molecule.

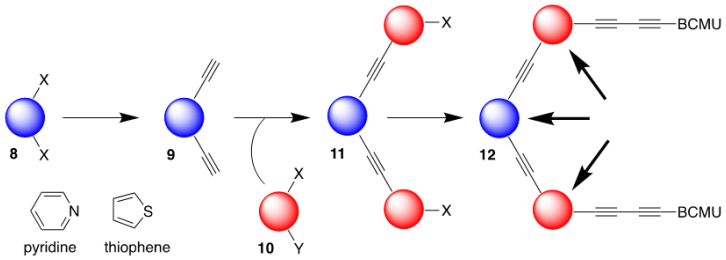


Figure 3: Proposed synthetic route to U-shaped diacetylenes.

This synthetic approach was found to be unacceptable, because all versions of structure **11** were very poorly soluble in all solvents.[16] Eventually, a revised approach in which the arm units containing the diacetylene-BCMU groups and an acetylene were prepared and then coupled with compounds of type **8**.[17] Though these synthetic procedures were complex, three examples of structure **12** were prepared and

characterized. Two of the three were shown to undergo polymerization with UV light and all three were found to polymerize thermally.

Polydiacetylenes- Strain and Impact Sensors

While the structural aspects of PDAs are interesting and potentially useful, their optical properties have attracted more attention and are features that can be incorporated into nanomachines. For example, we found that BCMU could be blended with the medical grade polyurethane Tecoflex.[18] The PDA phase separated into well-dispersed microcrystals. By monitoring the vibrational frequency of the carbon-carbon double and triple bonds using Raman spectroscopy, it was possible to quantify the strain experienced by the Tecoflex when it was stretched. This technique could be useful for predicting the failure of plastic components in structures ranging from medical implants to aircraft wings.

Related diacetylenes have been incorporated into polymers to create "smart packaging".[19] The diacetylene monomers can be dispersed into packaging materials without having a major effect on the material properties. With proper design, the packaging can sense physical impacts (Fig. **4**) or UV or gamma radiation.[20] Each of these stimuli will cause the diacetylenes to polymerize to highly colored PDAs and could be of use in detecting improper handling of food items, pharmaceuticals and many other items.

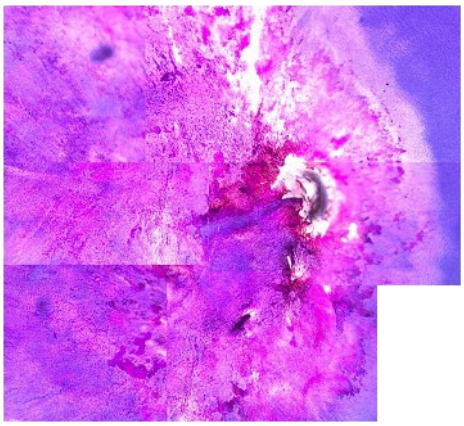
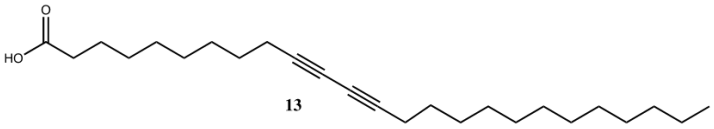


Figure 4: Photomicrograph of impact area on a sample from a height of 20.75 cm with a 100 g dart on a 20% 4-BCMUm blend with 60D Tecoflex®.

Another feature of PDAs that have received wide attention for the design of sensors is the color change from the initially formed blue color to red upon application of a stress. This stress can take many forms, including simple mechanical stress, heat, pH, or in more complex systems, bacteria or viruses. PDAs blended into polymer films can thus be used to record a thermal history of a package.[19] Finally, we have shown that PDA/polymer blends can be photopatterned with high resolution, potentially allowing the creation of smart labels that develop or change color in response to environmental signals.

Soft Nanomachines

Often, PDAs in the form of nanometer scale vesicles are used due to their higher sensitivity and the fact that they can be dispersed in water.[21] Liposomes are small spherical structures made from phospholipids (Fig. **5**); key components of cellular membranes. These have received a great deal of attention in the literature and have found use in commercial products. Rather than phospholipids, our work has made use of the diacetylene-containing fatty acid pentacosadiynoic acid, (PCDA, **13**). Like naturally-occurring phospholipids, PCDA is an "amphiphile". It contains a water-loving end and a water-hating end that allows it to self-assemble into a liposome (or more properly, an "ufasome" when using fatty acids) as shown in Figure **5**. The blue and grey circles are the hydrophilic carboxylic acid head groups, while the lines represent the hydrophobic hydrocarbon tails. As with polymerizable PDA crystals, adjacent diacetylene groups are aligned properly to undergo photopolymerization (represented by the curved red line). The PDA chains run across both the inner and outer layers of the membrane. The liposome is about 110 nm across and 6 nm wide, so it has a large water-filled interior cavity of nearly 100 nm in diameter. Hydrophilic molecules may be encapsulated in the inner core, while hydrophobic compounds migrate into the lipid bilayer. The exterior of the liposome can be decorated with biologically active compounds such as sugars, amino acids and their polymeric derivatives, polysaccharides and proteins. Even DNA segments and metal nanoparticles may be attached. Each of these modifications alters the properties and the potential uses of the liposomes. *Their small size, the variety of functionality possible and the ability of liposomes to respond to their environments allows us to define them as "soft nanomachines".*



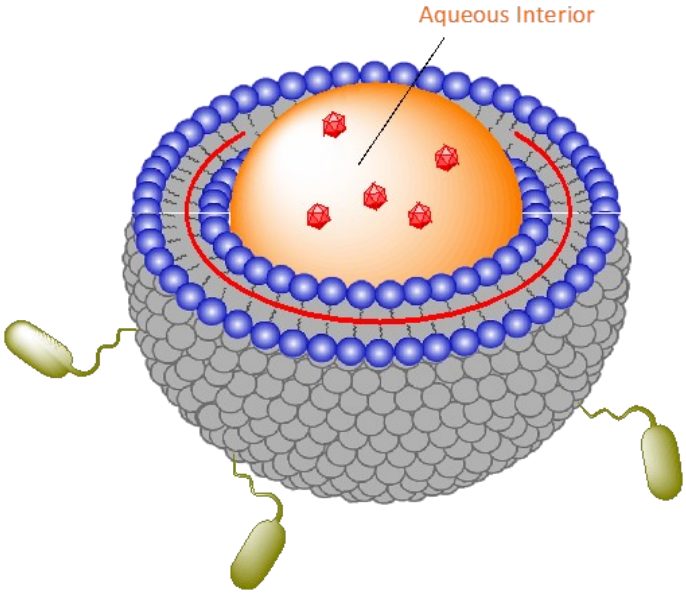


Figure 5: A representation of a PDA liposome.

We have made a number of contributions to this field. For example, by changing the hydrophilic head group from a carboxylic acid to a hydrazine, the liposomes change from blue to red as the solution is changed from neutral to basic. However, unlike the carboxylic acid systems, changing the pH to acidic also restores the blue color, making a reusable pH sensor. We also demonstrated that by placing a hydrophobic fluorophore in the bilayer, the sensors "turned on" fluorescence when converted to the red form but turned it off again in acid.[22]

Numerous research groups have developed biosensors based on PDA liposomes or related structures.[21] Based on some of the more common strategies, we developed an experiment for college educators to translate these concepts into an experiential learning experiment for the undergraduate laboratory.[23] Students derivatize PCDA to attach an amino acid (or amino acid derivative) to the head group. A small percentage of the derivatized PCDA is added to unmodified PCDA and liposomes prepared as usual. These deep blue solutions (Fig. **6**) are then exposed to extracts (lipopolysaccharides) from the cell walls of common food pathogens. The liposomes change from blue to red to an extent determined by head group and the pathogen. By choosing a selection of sensing systems, students can determine which pathogen is present.

In other work, we have developed new methods for the production of liposome biosensors that are more efficient and produce more

Figure 6: Liposomes prepared from 95% PCDA and 5% phenylalanine derivatized PCDA. 1) Treated with Salmonella; 2) Untreated 3) Treated with E. coli.

regularly sized vesicles than standard techniques.[24] Our method involves simple modifications of standard ink-jet printers and "printing" alcohol solutions of PCDA into water. The picoliter-sized droplets help to disperse the poorly soluble PCDA, allowing for efficient self-assembly without significant aggregation.

There are several issues that still must be addressed before PDA biosensors are widely used. For some applications, it is important that the biosensors be immobilized rather than freely dispersed in solution. To address this, we have incorporated them into a biopolymer known as alginate and spun the resulting solutions into fibers that could be used in wound dressings or tissue engineering scaffolds.[25] We have also looked at the challenges for deploying sensors into food processing facilities. One concern is that harmless residues from cleaning agents could result in false positives for bacteria. Our work helps to illustrate procedures to prevent this by describing the kinds of cleaning agents that are not compatible with the sensors.[26]

Figure 7: Alginate filaments containing blue PDA liposomes.

Future Prospects

Nanomachines built on the PDA nanovesicle platform have enormous potential for a wide range of applications. The work discussed in this manuscript focuses on the structural and sensing aspects of the structures, but there are other features that are being explored by the research community. The interior cavity can be used to transport drugs or other biologically active materials to specific locations within the body or the larger environment. Metallic nanoparticles can be encapsulated to allow for external control over liposome movement and oscillating magnetic fields can heat the structures to trigger the release of encapsulated contents on demand. Because the liposomes can be independently modified in so many ways, the variety of functions that may be incorporated allow for complex multifunctional devices. Their utility is only limited by our imagination.

Acknowledgements

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